FAILURE AT INDUCING RESISTANCE TO SCHISTOSOMICIDAL
DRUGS IN A BRAZILIAN HUMAN STRAIN OF SCHISTOSOMA MANSONI (1)

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SUMMARY

In this work, an attempt was made to induceng resistance to 3 schistosomicidal drugs in a Brazilian strain of S. mansoni, according to the Type II resistance induction scheme proposed by JANSMA et al. in 1977. Three attempts were unsuccessful. The parental generation treated with the drug during the immature stage of the worm was less susceptible to the chemotherapeutic agents than generations F_1 and F_2. A hypothesis is suggested as an explanation.

KEY WORDS: Schistosomiasis — Resistence to drugs;
Schistosoma mansoni — Brazilian human strain.

INTRODUCTION

In Brazil, Schistosomiasis mansoni is an important endemic disease, in several States[9], affecting about 10% of the Brazilian population. Many control program have been implemented, mostly employing chemotherapy as one of the main prophylactic methods to fight this parasitosis.

Mass treatment is currently being undertaken in Brazil using oxamniquine, with relative success, since the susceptibility of the Brazilian strains of S. mansoni to chemotherapeutic agents is reasonably good. Nevertheless several human strains resistant to oxamniquine have been described in Brazil[2,3,4,5,8]. Fortunately, the resistance phenomenon so far seems not to be an epidemiological problem since the cases of resistant worms are not numerous and, whenever tested, these strains showed susceptibility to praziquantel[1,4]. It is easy to foresee, however, the negative repercussions of a strain of S. mansoni resistant to oxamniqui-ne and/or other chemotherapeutic agent which might spread out in a given region.

Research on the mechanism of resistance development started with Rogers and Bueding, who, using mice infected in the laboratory, succeeded in experimentally induce resistance to hycanthone and oxamniquine in the progeny of worms treated with subtherapeutical doses of hycanthone[13].

JANSMA et al.[7] induced resistance to hycanthone and oxamniquine in the progeny of worms experimentally manipulated (further resistance) in 3 out of 5 strains of S. mansoni via three induction schemes. Type I resistance was obtained by means similar to those used by ROGERS & BUEDING[13]. Type II resistance was induced by treating mice harbouring the parental worm generation with subtherapeutic dosages of the drug at the time of the immature stage of infection, during the embriological development of the reproductive

system. Type III resistance was produced by infection of mice with cercariae of only one sex, followed by infection with cercarie of the opposite sex 2 to 58 weeks later, which didn't have any contact with the drug. The three types of resistance were limited to the progeny of the parental worms which showed susceptibility to the drugs used.

Theoretically, all these methods for producing resistance to S. mansoni could be inadvertently reproduced in the field, where mass treatment is frequently carried out indiscriminately. We believe that an experimental study on the resistance development possibility of in human strains of S. mansoni to certain drugs is very important, specially for determining the chemotherapeutic agent of choice for mass treatment of a population.

MATERIALS AND METHODS

1. S. mansoni strain

A Brazilian strain of S. mansoni, kept in our laboratory for several years, was used. It was isolated from a patient infected in Belo Horizonte, Minas Gerais, Brazil, and was named BH strain. In previous studies, this strain has already shown susceptibility to oxamniquine, praziquantel, niridazole and hyanthone[4].

2. S. mansoni hosts

Female albine mice (Mus musculus) weighing approximately 15 g at infection were used as definitive host. Intermediate hosts were laboratory raised albine Biomphalaria glabrata, descendant from molluscs captured in Belo Horizonte, Minas Gerais, Brazil.

3. Drugs and dosages

a — For induction:
oxamniquine — 50 mg/kg, single dose; oltipraz — 60 mg/kg daily for 5 days: praziquantel — 50 mg/kg daily for 5 days.

b — For effective treatment and assessment of susceptibility: oxamniquine — 100mg/kg, single dose; oltipraz — 125mg/kg daily for 5 days; praziquantel — 100mg/kg daily for 5 day; all drugs were given orally by gavage.

4. Induction scheme

Three groups of mice infected through the tail [10] with 70 cercariae of S. mansoni were treated with one of the drugs during the immature stage of infection, during the time of the embriological development of the genital organs of schistosomules of both sexes (between days 26 and 30, or on the 28th day after infection, according to the posology), in an attempt to induce drug resistance (Fig. 1).

Each group was then divided into 2 subgroups: trial and control. The animals in the trial subgroup were treated again, this time with therapeutic dosages of the respective drug, 60 days after the cercarian infection. After 20 days the animals of both subgroups were killed with a blow on the neck. Perfusion of the porta-mesenteric venous system and oograms of the small intestine were carried out [12,16].

The faeces of the animals in the control subgroup were used to obtain miracidia of F_1 generation, which were used for mass infection of B. glabrata [14]. The control subgroup was also used as standard for measuring the susceptibility of the trial subgroup.

After elimination of the cercariae from B. glabrata infected with F_1 generation, new groups of mice were inoculated through the tail with 70 cercariae.

Sixty days after the cercarian infection, the 3 groups of F_1 mice were divided into 2 subgroups: trial and control. The mice in the trial subgroup were treated with therapeutic dosages of the respective drug. Twenty days after the treatment, the animals in both subgroups were killed, and perfusion and oograms of the small intestine were performed [12,16].

A similar scheme was applied to F_2 generation.

5. Susceptibility criteria

a — Worm distribution.

The usual worm distribution pattern in the porta-mesenteric system obtained by perfusion was supplied by the control group. The finding of high percentages of worms in the

liver of animals in the trial subgroup was the evidence of the susceptibility of the worm to the schistosomicidal agent[11].

b — Percentage of oogram alterations

Oogram alterations were considered significant in the absence of one or more stages of immature eggs. This fact indicated interruption of oviposition, and hence, parasite susceptibility to the drug [11].

c — Percentage of worm reduction:

GONNERT & ANDREWS [6] estimated this rate by using the following formula:
Percentage of worm reduction =
$$= 100 \times \frac{\text{number of dead schistosoma}}{\text{number of live and dead schistosoma}}$$

d — Percentage of surviving worms:

This was obtained in accordance to the formula proposed by JANSMA et al [7]:
$$\text{Percentage of surviving worms} = \frac{T \times 100}{C}$$
where:

T is the average number of live worms per animal found in the trial group and C is the average number of live worms per animal found in the control group.

e — Reduction of parasite load

A significant reduction of the parasite load in the trial subgroup compared to the control subgroup indicates the susceptibility of the worm to the drug [11].

RESULTS

The percentage of males (Table I) was established in order to safely use the criterion of "worm distribution", because in the case of poorly balanced inoculations, with predominance of one sex, there is a tendency towards the concentracion of the worms in the liver and porta vein [15,17]. In this way, the data of the concentration of the worms in the liver since there was a tendency in the trial subgroup to present a higher percentage of males. After treatment it was more difficult to find

female than male worms due to their small size, desintegration and encapsulation in liver granulomas.

In 3 generations the mean, percentage of males in all control subgroups was 58.1% with a standard deviation of 9.7%, varying from 44.9% to 71.6% and thus compatible with the use of the "worm distribution" criterion for susceptibility analysis.

Results were very similar in all therapeutic groups. The parenteral generation of the worm was susceptible to the 3 drugs used (Table I). All trial subgroups presented large percentages of worms in the liver and porta vein, significant reduction of parasite load compared to the control subgroup, high rates of worm reduction, low rates of surviving worms and 100% change in oograms. These results occurred both in the parental generation and in F_1 (Table I), suggesting susceptibility of both generations, and therefore, failure of the attempt to induce resistance. Analysis of the F_2 generation showed results nearly identical to F_1.

An interesting and unexpected finding in the experiment was that the parental generation showed lower susceptibility than F_1 and F_2 generations, since in the former some surviving worms were still found after treatment, while in F_1 and F_2 surviving worms were practically inexistent.

DISCUSSION

The results showed that when submitted to JANSMA et al. Type II resistance induction scheme with the 3 schistosomicidal drugs used, contrary to expected, the susceptbiility of S. mansoni strain increased in successive worm generations. This not only indicated failure in the attempt to induce resistance but also broadened the possibilities for production of resistance to schistosomicides in S. mansoni strains. Many explanations can be offered for the fact that the parental generation present lower susceptibility than its progeny. The fact that the only difference in the manipulation of parental and progeny generations was the treatment of the parental generation during the immature stage of infection, lead us to raise the hypothesis that this treatment may have lowered the susceptibility of the parental generation, perhaps through an enzyma-

BH strain

↓

miracidia

↓

snails

↓

cercariae

↓

mice (parental generation)

↓

induction treatment
(26 to 30 days after cercarian infection)

trial subgroup

effective treatment
(60 days after cercarian infection)

perfusion and oogram

control subgroup

eggs   perfusion and oogram

miracidia

snails

cercariae

mice (F_1)

trial subgroup

effective treatment
(60 days after cercarian infection)

perfusion and oogram

control subgroup

eggs   perfusion and oogram

miracidia

snails

cercariae

mice (F_2)

trial subgroup   **control subgroup**

effective treatment   perfusion and oogram
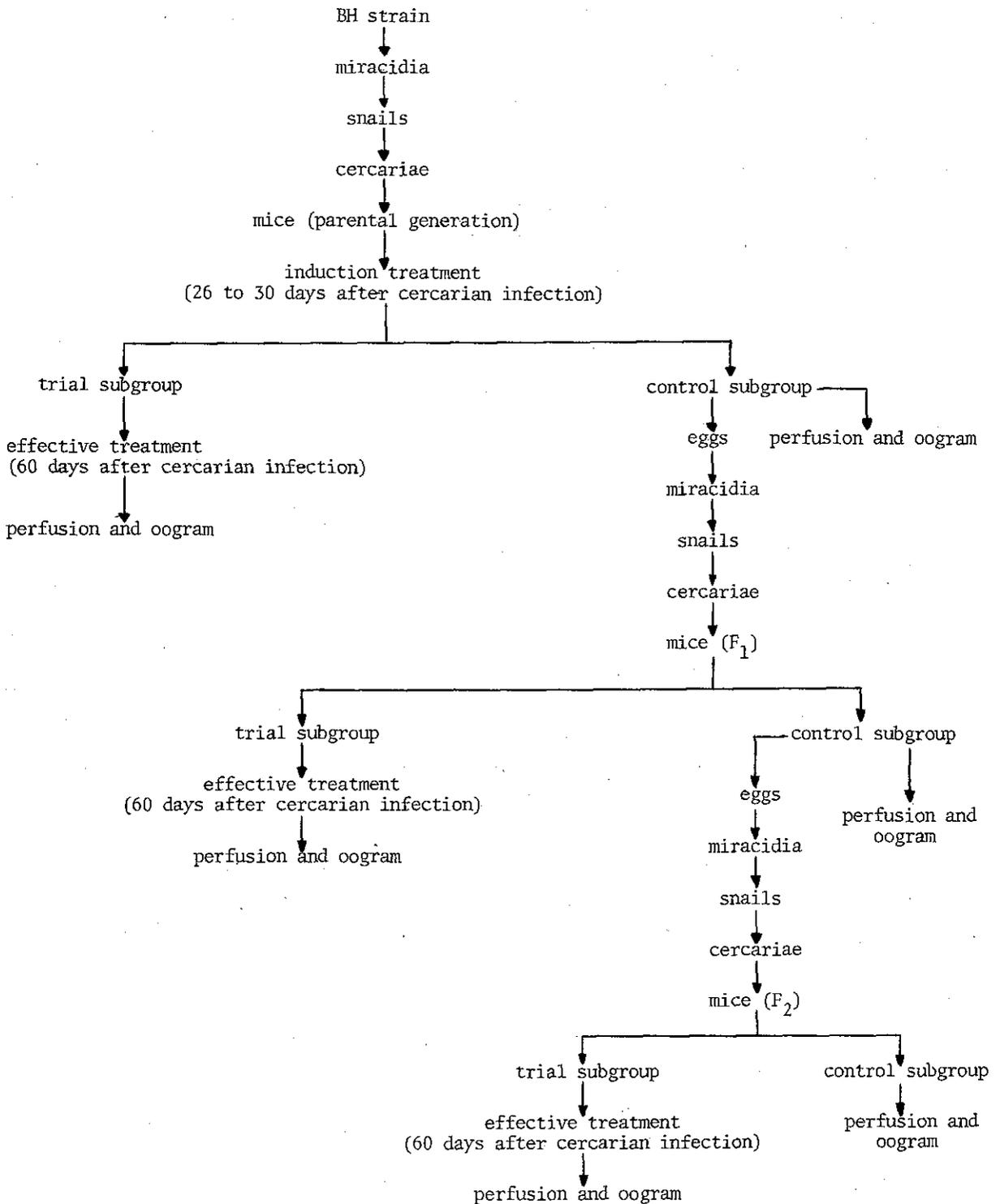(60 days after cercarian infection)

perfusion and oogram

Fig. 1 — Experimental model for invesigating the drug susceptibility of Schistosoma mansoni submitted to Jansma's Type II resistance induction scheme

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T A B L E I

Antischistosomal activity of drugs in mice experimentally infected with BH strain of Schistosoma mansoni, submitted to Jansma's Type II resistance induction schedule

Drug, Group and Progenie			Number of examined mice	Percentage of worm in			Mean worm burden	male	Percentage of		oogram changes
				Mesenteric vessels	Portal vein	Liver			worm reduction	surviving worms	
OLT	P	T	8	0,0	0,0	100,0	8,5	69,0	80,9	16,0	100,0
		C	6	40,7	11,1	48,2	13,5	59,2	0,0	—	0,0
	F₁	T	5	0,0	0,0	100,0	8,6	74,4	100,0	0,0	100,0
		C	2	76,9	17,9	5,1	19,5	51,3	0,0	—	0,0
	F₂	T	8	0,0	0,0	100,0	11,6	89,2	100,0	0,0	100,0
		C	5	55,6	11,1	33,3	28,8	70,8	0,0	—	0,0
PRZ	P	T	10	0,0	0,0	100,0	6,3	71,4	85,7	12,0	100,0
		C	10	83,1	11,0	5,9	13,6	44,9	0,0	—	0,0
	F₁	T	9	0,0	0,0	100,0	12,3	72,9	100,0	0,0	100,0
		C	6	57,9	27,8	14,3	22,2	57,9	0,0	—	0,0
	F₂	T	10	0,0	0,0	100.0	4,7	74,5	100,0	0,0	100,0
		C	9	59,7	25,4	14,9	17,1	61,7	0,0	—	0,0
OXM	P	T	8	0,0	5,3	94,7	4,6	56,8	64,5	8,0	100,0
		C	7	73,6	16,4	9,7	20,0	45,0	0,7	—	0,0
	F₁	T	7	0,0	2,4	97,6	12,0	84,5	91,7	0,04	100,0
		C	7	58,5	26,5	14,8	27,9	61,0	0,0	—	0,0
	F₂	T	10	0,0	0,0	100,0	9,0	79,4	100,0	0,0	100,0
		C	10	51,8	30,5	17,7	14,1	71,6	0,0	—	0,0

Obs.: OLT = Oltipraz; PRZ = Praziquantel; OXM = Oxamniquine; P = Parental generation; F₁ and F₂ = Progenies; T = Trial subgroup; C = Control subgroup.

tic induction process. This observation may be of value and opens new fields of research on the mechanism of producing resistance to schistosomicidal agents in S. mansoni.

Furthermore, failure in the attempt to induce resistance to schistosomicides in a Brazilian strain of S. mansoni is a good result, because it suggests a low potential of this strain to become resistant, in a country where mass treatment situations favour the development of such a resistance. Nevertheless more trials must be undertaken to evaluate this potential in relation to other brazilian strains, applying different induction schemes.

CONCLUSIONS

a. The JANSMA et al. Type II resistance induction scheme failed to induce resistance to schistosomicidal drugs in the Brazilian strain of S. mansoni (BH from Minas Gerais).

b. The parental generation of S. mansoni treated with schistosomicidal drugs during the immature stage of infection showed less susceptibility than its progeny. Further research must be carried out in order to determine the effect that this type of exposu-

re may have on the susceptibility of the worm.

RESUMO

Insucesso na indução de resistência a drogas esquistossomicidas em uma cepa brasileira humana de Schistosoma mansoni

No presente estudo, realizou-se uma tentativa de indução de resistência a 3 drogas esquistossomicidas em uma cepa brasileira de S. mansoni, segundo o esquema de indução de resistência tipo II preconizado por JANSMA et al. em 1977. Houve insucesso nas 3 tentativas realizadas. A geração parental tratada com a droga durante o estágio imaturo do verme mostrou-se menos suscetível aos quimioterápicos do que as gerações F₁ e F₂ do verme. Uma hipótese é levantada para a explicação do fato.

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